THE ROLES OF DARKNESS AND LIGHT IN THE PHOTO–PERIODIC RESPONSE OF THE TESTES OF WHITE–CROWNED SPARROWS [1]

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During the past quarter century a substantial amount of investigation has been directed towards the so-called photoperiodic response in birds, whereby it is possible to obtain an unseasonal gonadal development by increasing the length of the daily photoperiod. These investigations have been summarized very adequately by Burger (1949) and Benoit (1950). A very interesting and useful approach to an understanding of the basic mechanism involved in this phenomenon is the use of interrupted daily photoperiods. The first use of this experimental approach was apparently that of Benoit (1936). In his experiments young male ducks were maintained in dim essentially non-stimulatory light except for the experimental periods of exposure to light of stimulatory intensity when they were transferred to a light room. Benoit found that ducks exposed to 10.5 minutes of light per hour for 14 hours per night (ca. 2.5 hours total light) for a period of 16 days showed a generally greater testicular response than those which received single periods of 2.5 hours per night. Burger *et al.* (1942) obtained testicular responses in starlings (*Sturnus vulgaris*) given ten hours of light daily as a period of six hours continuous light plus eight hours of alternating 5-second light and 5-second dark periods. The entire treatment had a duration of 14 hours daily; the testicular responses were the same as those of starlings which received daily 14-hour periods of continuous light. Uninterrupted 10-hour light periods are essentially non-stimulatory in this species.

Kirkpatrick and Leopold (1952, 1953) have recently shown with bobwhite quail (*Colinus virginianus*) that, although 10 hours of continuous light daily for 37 days failed to evoke a gonadal response, the same total duration of light, but with 15–60 minutes used as an interruption of the dark period, resulted in gonadal activation. It was found that the percentage of full sexual activity after 64 days of such treatment was directly proportional to the length of the interrupting light period and inversely proportional to the length of the longer dark period. These results prompted the investigators to conclude that both the light period and the dark period are involved in sexual activation in this species, that the dark period is an inhibiting factor, and that the duration of the dark period is a major controlling factor in the response. Jenner and Engels (1952) in experiments with male slate-colored juncos (*Junco hyemalis*) and white-throated sparrows (*Zonotrichia albicollis*) have

likewise found that it is possible to obtain testicular development with a total of 10 hours of light per day when 1¾ hours of it were used as an interruption in the middle of the dark period. Ten-hour daily photoperiods are essentially non-stimulatory in these species. These investigators have concluded that in the photoperiodic response of these species there is a (p. 354) ". . . critically important dark-dependent phase." This is somewhat difficult to rationalize in light of the testicular responses obtained by Winn (1951) and Wolfson (1952a) in the same species with exposure to continuous light.

We have recently completed a series of experiments in which we have exposed male white-crowned sparrows (*Zonotrichia leucophrys gambelii*) to several patterns of intermittent lighting. The results of these experiments are in accord with the data obtained by Kirkpatrick and Leopold (1952, 1953) and Jenner and Engels (1952); however, they lead us to feel that the assumption of an essential or active role by the dark period in the photoperiodic response is not necessary. It is the purpose of this paper to describe the results of our experiments and to present an hypothesis which rationalizes the available data on avian gonadal responses to intermittent lighting.

METHODS

Our experimental birds were captured with Japanese mist nets from the population of *Zonotrichia leucophrys gambelii* which winters in the Snake River Canyon of southeastern Washington. On February 14, after an adequate period of con-

TABLE I

Resumé of experimental conditions

Group	Number of males	Mean aviary temperature °C.	Daily light treatment		
			Schedules	Total hours of light	Total hours of treatment*
A	8	20	0700–1700 continuous	10	10
B**	12	6	0554–1806 continuous	12.2**	12.2**
C	7	21	0300–2100 continuous	18	18
D	10	16	0800–1630 and 2330–0100	10	17
E	15	20	Continuous 0710 to 1650 plus one minute each half-hour from 0300 to 0700 and from 1700 to 2100	10	18
F	13	21	Nine 1.11-hour periods separated by 1-hour periods of darkness (0300 to 2100)	10	18

* Time elapsed from the beginning of the first (or only) period of light to the end of the last (or only period) of light.

** Wild controls; mean daylight hours February 14 to March 16 including civil twilight.

ditioning to cage life, five groups were placed in separate aviaries and subjected to the experimental conditions indicated in Table I. Additional wild birds (group B of Table I) were collected and examined through the course of the experiments. Each group contained a few females; because of the small numbers and the variability in responses in this sex, the data from these are not included in this report. The

illumination was provided by incandescent lamps at intensities of 30–40 foot candles within each cage. This is substantially in excess of the minimum required to induce a gonadal response. All lights were operated automatically. Groups A and E were kept in separate light-insulated compartments in the same constant-condition aviary. The other three experimental groups were maintained in widely separated indoor aviaries in which thermographic recording was employed to ascertain mean temperatures.

The birds in all experimental groups were kept in flight cages ($12 \times 24 \times 18$ inches), about six per cage. Food, water, and grit were provided *ad libitum*. Food consisted of red millet seed supplemented by a vitamin- and mineral-enriched poultry mash prepared by the Department of Poultry Science of the State College of Washington.

The treatments, as indicated in Table I, were continued for 29–30 days. Body weights and observations on the plumage were recorded weekly. At the conclusion of the experimental period the birds were killed and autopsied. The gonads were placed in alcohol-formaldehyde-acetic acid fixing mixture, and after several

TABLE II

Testicular weights

Group	Number	Arithmetic mean (mg.)	Range	Geometric* mean (mg.)
A	8	1.2	0.6–1.6	1.2
B	12	3.9	3.0–6.0	3.8
C	7	190	79–250	170
D	10	8.2	3.0–25	6.8
E	15	87	17–210	65
F	13	170	35–310	140

* Obtained by logarithmic transformation of testicular weights.

days, to allow thorough impregnation, were weighed on a Roller-Smith Precision Balance. For histologic examination, one testis from each male was imbedded in paraffin, sectioned at eight micra, and stained with acid hematoxylin and yellowish eosin.

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RESULTS

The responses to the various treatments, in terms of testicular weights, are given in Table II. Unfortunately it was not possible to maintain the temperature of the aviary containing group D at the same level as that of the other experimental groups. Had this been possible, the response of the testes in group D would doubtless have been somewhat greater (Farner and Mewaldt, 1952) although it is highly improbable that the degrees of development of groups C, E, and F would have been approached. The test of Cochrane and Cox (Snedecor, 1946) for significance of differences between means involving samples of different sizes and differences in variance has been applied to all possible pairs of arithmetic means.

All differences are highly significant ($P = 0.01$) except those between the means of B–D, C–E, C–F, and E–F; however, the differences between the means of C–E and E–F are significant at the 5 per cent level. Statistical analyses of the logarithms (Bartlett, 1947) of testicular weights indicate that the differences between all possible pairs of geometric means except C–F are highly significant.

In our histologic studies we have first compared a normal series of wild and captive birds with the description of testicular development in this subspecies as presented by Blanchard and Erickson (1949). We found their description to fit our material very well, with the exception of the distinction between stages I and II (see also Blanchard, 1941) which is based on the absence of cells of Leydig in the former. We have consequently adopted Blanchard's (1941) stages in the analysis of our material although we do not separate stages I and II. The results of our histologic studies are presented in Table III. It should be noted that the 10-hour controls (group A) showed no development beyond the normal winter resting condition,

TABLE III

Testicular development

Group	Number examined	Stages* of development
A	8	8 in I–II
B	2**	2 in III**
C	7	4 in VI; 3 in VII
D	10	7 in III; 3 in IV
E	15	3 in IV; 5 in V; 5 in VI; 2 in VII
F	13	1 in V; 3 in VI; 9 in VII

* According to Blanchard (1941).
** Of the 12 birds in this group only two were examined histologically. Histologic examination of another and more extensive series of testes from birds collected in the wild reveals that all were in stage III on March 16.

whereas the wild controls, subjected to the normal spring increments in daily photoperiod, had reached stage III by March 16. In general the responses ascertained histologically closely parallel the responses indicated by testicular weights.

Discussion

The nature of the mechanism of the photoperiodic gonadal response in birds is by no means completely understood. That the anterior pituitary gland is an essential part is patent; much of the pertinent evidence has been summarized by Burger (1949) and Benoit (1950). The ingenious experiments of Benoit (1937, 1938a, 1938b; Benoit and Kehl, 1939; Benoit and Ott, 1944) have shown further that nonocular reception of light may be involved, and that the hypothalamus is quite likely an important part of the mechanism. Wolfson (1952a) has shown that the gonadotropic effect of daily photoperiods, at least between certain minimum and maximum lengths, is a direct function of photoperiod length, and that the daily increments appear to be summated in exerting their effect. A consideration of the results of our experiments suggests the possibility that this light-stimulated mechanism is of

such nature that there is involved a process which becomes active almost immediately after the beginning of the photoperiod and which has a persistent "carry-over period" of activity after the end of the photoperiod. We envision the effective duration of this "carry-over period" to be of the order of a fraction of an hour to several hours, doubtless a function of the duration and nature of the preceding photoperiod. We likewise envision its characteristics as differing in different species. Our hypothesis suggests further that the rate of testicular response would be a direct function of the summated daily gonadotropic effects of the photoperiods and the "carry-over periods." Under natural conditions, of course, there would be one of each per day. Although our data provide no direct indication of the possible physiologic basis for such a "carry-over period," several suggestions may be made in order to suggest the plausibility of the hypothesis. It is possible that the actual synthesis of gonadotropins could involve a light-dependent reaction. The "carry-over period" would then be the period of the dissipation of the gonadotropins down to an ineffective level. There is also the possibility that the actual transfer of the pituitary gonadotropins into the blood stream involves a light-dependent reaction. This is in accord with recent evidence of Benoit *et al.* (1950) which suggests that the anterior pituitary of the duck tends to release gonadotropins during light periods and to retain them during darkness. Should this be the case, the "carry-over period" might be the time required for blood gonadotropins to fall below a threshold concentration. There is further the possibility that the light-dependent reaction may be in the hypothalamus where it produces a substance which exerts a humoral control over the anterior pituitary.

With respect to the results of our experiments, this hypothesis can account for the difference between groups A and D as the effect of the additional "carry-over period" in D. The treatment and responses in group D were quite similar to those described by Jenner and Engels (1952) for white-throated sparrows and slate-colored juncos. The differences between D and E could be attributed to a longer period of gonadotropic effect in the latter because of the 17 additional "carry-over periods." The differences between E and F could be the result of the greater activity of a "carry-over period" which follows a one-hour photoperiod than of one which follows a one-minute photoperiod. Further investigations are needed to ascertain more precisely the functional relationship between the activity of a "carry-over period" and the duration of the preceding photoperiod. The data of Kirkpatrick and Leopold (1952, 1953) actually fit this hypothesis quite nicely. The inverse relationship between the length of the longer dark period and the gonadal response might be explained as the attainment of the maximum effects of two "carry-over periods" by optimum timing of the interrupting light. The direct relationship between the length of the interrupting light period and gonadal response, as reported by Kirkpatrick and Leopold (1953), conforms with our suggestion that the activity of a "carry-over period" is some direct function of the length of the preceding photoperiod. We feel that this hypothesis also rationalizes the results obtained with interrupted photoperiod by Benoit (1936) on ducks, and quite possibly also those of Burger *et al.* (1942). In the latter case it would be necessary to assume that very short photoperiods (5 seconds) were followed by "carry-over periods" of effective gonadotropic activity in the order of at least a few seconds duration.

It appears that this hypothesis might also be applicable to the mechanism of light-stimulated increase in egg production in the domestic fowl since it has been shown by Dobie *et al.* (1946), Staffe (1950, 1951), and Weber (1951) that various patterns of interrupted light give greater responses than the same amount of light as a single photoperiod. It appears that the reasoning in our hypothesis may be quite similar to that of Staffe (1951) although he is concerned primarily with the mechanism of the stimulation of increased pituitary activity with respect to maintaining a high rate of egg production. The response of sticklebacks (*Gasterosteus aculeatus*) to intermittent light as observed by van den Eeckhoudt (1947) suggests that such a mechanism may exist in this species. On the other hand it appears unlikely that the gonadal responses obtained in photoperiodic investigations of sheep (Yeates, 1949; Hart, 1950) and ferrets or mink (Hammond, 1951, 1952; Hart, 1951) can indicate such a simple rationalization for the mammals with photoperiodically-regulated reproductive cycles. In this respect it should be noted that Hammond (1952) is of the opinion that the mechanisms are basically different in birds and mammals.

Apparently in most, if not all, avian species a refractory period develops following a cycle of gonadal development. During this refractory period an increase in daily photoperiod does not evoke a gonadal development. The available evidence (Damsté, 1947; Wolfson, 1952a, 1952b) suggests that short daily photoperiods, or long dark periods, are necessary to terminate the refractory period. However, once the refractory period has been terminated, our hypothesis suggests, apparently unlike the thinking of Kirkpatrick and Leopold (1952) and Jenner and Engels (1952), that the daily dark period makes no positive contribution to the mechanism of response, although, of course, the gonadotropic effect of a "carry-over period" may be exerted during the dark period. The differences may be largely semantic; we feel, however, that our hypothesis is basically different and represents a more logical rationalization of the available data. Our hypothesis is then consistent with the suggestion of Hammond (1953) that darkness may have no active role in avian photoperiodism.

Kirkpatrick and Leopold (1952) and Jenner and Engels (1952) have emphasized possible analogies between photoperiodism in birds and in plants on the basis of their conclusions that darkness has some essential role in avian photoperiodism and the rather extensive body of evidence (Leopold, 1951; Lang, 1952) of the existence of dark-dependent reactions important in photoperiodism in plants. We feel, however, that the greatest of caution must be exerted in any serious development of such an analogy on the basis of the presently available information on birds, since there appears to be no reason to assume that the effect of darkness in the photoperiodic mechanism is anything other than that of the cessation of a process requiring light.

The hypothesis of the "carry-over period" is proposed simply as one possible rationalization of the available information on the nature of the mechanism of photoperiodism in birds. Its extension and its ultimate acceptance will rest on the results of future investigations. In consideration of the available evidence, however, we feel that two arguments favor this hypothesis: (1) it rationalizes the available information, and (2) as we have argued in our preliminary report, it is in accord with the Law of Parsimony as the simplest apparent rationalization.

SUMMARY

1. Experiments were conducted with males of a migratory race of white-crowned sparrows to ascertain the nature of response to interrupted photoperiods of several patterns.

2. The results were as follows:

(a) Testes of birds on a 10-hour continuous daily photoperiod showed no response during the 29–30 days of the experiment.

(b) Wild controls, exposed to the naturally increasing daily photoperiods from mid-February to mid-March had just begun vernal spermatogenic development and showed slight increases in testicular weight.

(c) Exposure to continuous 18-hour daily photoperiod resulted in complete spermatogenesis, or nearly complete spermatogenesis, and a marked increase in testicular weights.

(d) Daily exposure to 10 hours total light divided into two periods, 8½ hours during the day and 1½ hours in the middle of the night, resulted in a considerably greater degree of gonadal development than the wild controls.

(e) Daily exposure to 10 hours total light, as 9 hours 40 minutes of continuous light plus one minute light per half-hour for 4 hours before and after the continuous period, resulted in a markedly greater response than in (d).

(f) A still greater response was obtained with daily exposure to 10 hours total light divided into nine 1.11-hour periods equally spaced through the course of 18 hours. Spermatogenesis was as completely developed as in (c). Statistically there is no significant difference between the response in testicular weight of this group and the group subjected to continuous 18-hour daily photoperiods.

3. These results suggest to us that the light-stimulated mechanism involves a light-dependent process which responds quickly at the beginning of a period of light and soon produces a sufficient quantity of an essential substance to allow a persistent gonadotropic effect after the cessation of light. We refer to the period of gonadotropic effect after the cessation of light as the "carry-over period." It is our opinion that the assumption of an active or essential role with respect to the dark period, is not necessary in rationalizing the available information on photoperiodism in birds.

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